Measurement of cardiomyocyte diameter in the isolated rat heart using diffusion-weighted ^{1}H-MRS: Changes with ischaemia

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Introduction

Cells regulate their volume in response to changes in osmotic pressure gradients across the sarcolemma. However, if the volume regulating process is not fast enough to extrude the surplus water from the cell, swelling and damage may occur. It has been suggested that the extent of cell swelling during ischaemia is directly related to the severity of an ischaemic insult, making cell volume a possible indicator of injury. The aim of this work was to determine whether the average ventricular myocyte diameter can be estimated in the perfused and the ischaemic rat heart by measuring the ADC of cytosolic taurine molecules at a range of diffusion times. Data were analysed according to the method of Mitra and colleagues (1).

Methods

General

Hearts from rats with a mean body weight of 345 ± 24 g (n = 9) were excised and perfused in the Langendorff mode with modified Krebs-Henseleit buffer containing 5 mM acetate, 1.8 mM pyruvate and 0.2 mM lactate as substrates. A water-suppressed slice-selective STEAM sequence was used with a TE of 20 ms. The ADC of intracellular taurine was obtained from a 3 mm axial slice through the ventricles.

Myocyte diameter in the perfused heart

One group of hearts (n = 5) was used for the measurement of the average cardiomyocyte diameter during KCl-arrest perfusion. The water-suppressed slice-selective STEAM sequence was used with a TR of 4 s, and 17 TMs ranging from 40-1500 ms. The T_1 of taurine was sufficiently long to obtain adequate signal, even at a TM of 1500 ms (data not shown). Five *b*-values were used with the strength of the diffusion gradients adjusted such to yield similar *b*-values at each TM time, generally ranging from 9-1466 s/mm^2.

Myocyte diameter in the ischaemic heart

The second group of hearts (n = 4) was used to measure the ADC of taurine after 30 min of total global ischaemia. Two measurements were performed with mixing times of 40 ms and 800 ms, to increase the sensitivity of the taurine ADC to restriction effects. The TR was 3 s and 16 averages were acquired for each spectrum. Five *b*-values were used, ranging from 37-1408 s/mm^2.

Results

During perfusion, the taurine ADC in all hearts decreased with increasing TM, indicating restriction caused by the sarcolemma (Figure 1). Using these data, the average diameter of cardiomyocytes was calculated to be 40 ± 6 μm. After 30 min of ischaemia, the taurine ADC was (0.28 ± 0.03) × 10^{-3} mm^2/s at a TM of 40 ms, and (0.11 ± 0.01) × 10^{-3} mm^2/s at a TM of 800 ms (data not shown). From these data, the average cardiomyocyte diameter during ischaemia was estimated to be 27 ± 5 μm, suggesting that the myocyte diameter decreased by 32% over 30 min of ischaemia.

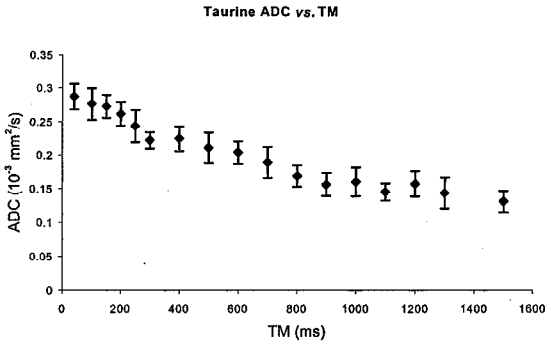

Figure 1: *Taurine ADC as a function of TM.*

Discussion & Conclusions

The decrease in the taurine ADC with increasing diffusion times suggests that restriction increased the longer the taurine molecules were allowed to diffuse. These restriction effects were probably due to elastic interactions of the taurine molecules with the sarcolemma (2).

The model to estimate a compartment size using ADCs as a function of the diffusion time has been proposed by Mitra and colleagues (1992), and applied by others to skeletal muscle and plant tissue (2, 3). Here, the average cardiomyocyte diameter was found to be 40 ± 6 μm, larger than published values of single isolated rat cardiomyocytes of 27 ± 1 μm (4, 5). However, hearts perfused with crystalloid buffer are known to be highly oedematous (6). Hence the average myocyte diameter in the perfused heart is much larger than the '*in vivo*' diameter. The myocyte diameter of 27 ± 5 μm in the ischaemic heart is identical to published values of 27 ± 1 μm in isolated rat cardiomyocytes (4, 5). At long TM times, the decreased taurine ADCs could be attributed to restriction effects caused by the sarcolemma. Because cells shrank at the start of ischaemia due to the loss of perfusion pressure, restriction increased, resulting in a decrease in the ADC.

This is the first time that a metabolite ADC has been used to measure the cardiomyocyte diameter in the intact perfused and ischaemic rat heart.

Acknowledgements

The authors are grateful to Mr. D.E. Befroy and Mr. P.J. Cassidy. CL was supported by a fellowship from the EC. Support from the British Heart Foundation is gratefully acknowledged.

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